

have canceled claim 3 and rewritten the claim as claim 11 as requested by the Examiner. Thus, upon entry of the amendment, claims 1, 2 and 4-11 are under examination.

**I. REJECTIONS UNDER 35 U.S.C. §101 and 112**

Applicants respectfully request that the rejection of claims 1 and 9 under 35 U.S.C. §101, be withdrawn. Applicants have amended claim 1, from which claim 9 depends, to recite that the polynucleotide encodes a desired property or a biological activity. The Office Action states that the polynucleotide to be mutated had no specific use, e.g., coding for a useful product.

The present invention provides methods for producing a mutant polynucleotide encoding a desired property or desired biological activity, as defined by amended claim 1, by blocking or interrupting a polynucleotide synthesis or amplification process by contacting the polynucleotide with one or more agents that block or interrupt polynucleotide synthesis or amplification process to provide a plurality of recombinant polynucleotides due to said polynucleotides being in various states of synthesis or amplification and subjecting one or more of the recombinant polynucleotides to an amplification procedure.

In another embodiment, the invention, as defined by claim 2, provides methods for producing a mutagenized polynucleotide encoding a polypeptide having a desired property by blocking or interrupting a polynucleotide synthesis or amplification process using one or more of such agents so as to provide a plurality of single or double-stranded polynucleotides; denaturing the plurality of different single or double stranded polynucleotides to produce a mixture of single-stranded polynucleotides; incubating all or part of the single stranded polynucleotides with a polymerase under conditions which result in annealing of the single-stranded polynucleotides at regions of shared homology and under conditions which promote synthesis of double-stranded polynucleotides so as to form mutagenized polynucleotides; and expressing at

least one mutant polypeptide from the mutagenized polynucleotides that has a desired characteristic.

The invention methods provides a non-natural means of generating a plurality of different mutagenized double stranded polynucleotides from a template polynucleotide that can be used to obtain a mutant polypeptide (e.g., one not found in nature as a wild-type) possessing a desired characteristic. For example, the mutagenized double-stranded polynucleotides can be inserted into vectors and expressed as polypeptides that can be assayed to identify those having altered function, such as greater enzymatic activity at a particular pH, or increased thermostability.

Those of skill in the art understand the importance of natural homologous recombination. In sexually reproducing organisms this occurs during meiosis when double stranded DNA divides and one strand of the original double stranded molecule recombines with another single strand (i.e., provided by another organism). This process of natural 'genetic shuffling' provides a method for increasing genetic diversity that aids in adaptation and survival.

Similarly, in the invention methods, as defined by claims 1 and 2, non-natural methods are provided by which the sequences of polynucleotides may be 'shuffled,' resulting in genes that encode polypeptides with enhanced or novel characteristics.

The present invention discloses a method for shuffling homologous regions within and between polynucleotides. Accordingly, the invention provides methods for producing polynucleotides that express polypeptides that can be screened to detect altered function as compared to corresponding polypeptides from parent polynucleotides. Such altered function may be increased thermostability, greater structural integrity, and the like. Such a method has further practical importance to the biotechnology community for generating biomolecules, that are more desirable than their wild-type counterparts for use in commercial manufacturing

processes, which are typically conducted under conditions that denature biomolecular structure or otherwise alter biomolecule function.

Claims 1 and 9 provide a credible and substantial utility, as discussed above. Accordingly, Applicants respectfully request withdrawal of the rejection.

Applicants respectfully request that the rejection of claims 1-9 under 35 U.S.C. §112, first paragraph be withdrawn. Applicants have provided a process for producing mutant polynucleotides which express a useful mutant polypeptide by a series of steps. For example, the steps may include:

- (a) producing polynucleotides by interrupting a polynucleotide amplification or synthesis process with a means for blocking or interrupting the amplification or synthesis process and thus providing a plurality of smaller or shorter polynucleotides due to the replication of the polynucleotide being in various stages of completion
- (b) denaturing the resulting single- or double-stranded oligonucleotides to produce a mixture of single-stranded polynucleotides,
- (c) incubating a said polynucleotides mixture of single-stranded polynucleotides with a polymerase under conditions which result in annealing of said single-stranded polynucleotides at regions of homology between the single-stranded polynucleotides and under conditions which promote synthesis of double stranded polynucleotides and thus forming mutagenized polynucleotides;
- (d) optionally repeating steps (b) and (c);
- (e) expressing at least one mutant polypeptide from said mutagenized polynucleotide; and
- (f) screening said at least one mutant polypeptide for a useful activity.

In a preferred aspect of the invention, the means for blocking or interrupting the amplification or synthesis process is by utilization of uv light, DNA adducts (as seen in new claim 11), DNA binding proteins, heat or cold, for example. Such steps were clearly delineated in US Patent 5,965,408, as well.

Applicants have provided prophetic examples, as noted by the Examiner, which specifically outline steps required to fully teach one of skill in the art to make and use the claimed invention. The method of the present invention incorporates any means which slows or interrupts polynucleotide synthesis. Such a means can be refractory to removal, such as intra-strand dimer formation induced by UV radiation, or easily removable, such as the DNA intercalator ethidium bromide. Applicant submits that the teachings of the presence of an adduct on the template strand of duplex DNA to induce termination of a growing polynucleotide chain during DNA synthesis without precluding the use of such a polynucleotide as a primer for further DNA synthesis reactions is fully supported in the application as filed. For example, cyclobutane dimers and (6-4) photoproducts are the most prevalent photoproducts resulting from UV C or UV B irradiation of DNA. These photoproducts interrupt polynucleotide synthesis because most DNA polymerases are unable to traverse such adducts. Termination of synthesis results in a duplex nucleic acid consisting of: 1) a short polynucleotide representing premature synthesis termination and which does not contain an adduct and 2) a template strand which contains a photoadduct. Upon denaturation of the duplex, the short single-stranded polynucleotide is available to anneal to an appropriate template present in the heterogeneous DNA population. The 3' hydroxyl end of the single-stranded polynucleotide is unaffected by the photoadduct and is, therefore, capable of supporting initiation of synthesis when annealed to a complementary template. Moreover, the photoadduct-containing template strand is available to act as a template for further synthesis reactions. In effect, the process described in the previous example and claimed in the present invention results in the random redistribution of polynucleotides and templates, ultimately giving rise to unique nucleic acid sequences. Further, Applicants have actually reduced this invention to practice and would be willing to submit results in a declaration under section 1.132 by the inventors if requested by the Examiner.

In one aspect, the present method relies on the ability of agents to interrupt successive cycles of DNA synthesis resulting in a population of shortened polynucleotides. These shortened polynucleotides are available to anneal to, and initiate synthesis from, additional

complementary templates contained in the heterogeneous sample. For example, treating a sample containing duplex DNA molecules with a limiting amount of an interstrand cross-linking agent will result in a population of molecules cross-linked at various locations within the duplex. Upon partial denaturation, regions of single-strandedness will be accessible to primers which promote DNA synthesis. Successive rounds of amplification and random priming ultimately results in full-length, chimeric duplex molecules which encode polypeptides with unique properties. Applicants submit that these teachings are clear in the application as filed and therefore request that the rejection be withdrawn.

**II. REJECTIONS UNDER 35 U.S.C. §102**

Claims 2,6-8, and 10 stand rejected under 35 U.S.C. §102 as allegedly anticipated by Pues, et al. Applicants respectfully traverse this rejection. The 1997 publication date of Pues et al. is after Applicants' priority date of July 9, 1996, therefore Pues et al. is not even available as "prior art". Accordingly, Applicants request withdrawal of this rejection.

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**CONCLUSION**

In summary, for the reasons set forth herein, Applicants maintain that claims 1, 2, and 4-11 clearly and patentably define the invention, respectfully request that the Examiner reconsider the various grounds set forth in the Office Action, and respectfully request the allowance of the claims which are now pending.

If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative can be reached at (858) 677-1456. Please charge any additional fees, or make any credits, to Deposit Account No. 50-1355.

Respectfully submitted,

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**Version with Markings to Show Claim Amendments**

1. (Twice amended) A method for producing a mutant polynucleotide[s] encoding a polypeptide having a biological activity or a desired property comprising:  
blocking or interrupting a polynucleotide synthesis or amplification process [in a recombinant cell system by treating the cells] by contacting a polynucleotide with one or more agents that block or interrupt synthesis or amplification of [a] the polynucleotide wherein the agent is selected from the group consisting of UV light, one or more DNA adducts, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerase, cold or heat, chain terminators, [and] polymerase inhibitors [or] and poisons [to provide a plurality of different recombinant polynucleotides due to said polynucleotides being in various states of synthesis or amplification,] and subjecting said [recombinant] polynucleotides to an amplification procedure [to amplify one or more of the polynucleotide or polynucleotides] to provide a mutant polynucleotide.

2. (Amended) A method for producing a [recombinant] mutagenized polynucleotide encoding a polypeptide having a desired property, said method comprising:

(d) [producing a plurality of different oligonucleotides by] blocking or interrupting a polynucleotide synthesis or amplification process with at least one member selected from the group consisting of UV light, one or more DNA adducts, DNA intercalating agents, chain terminators, and/or polymerase inhibitors or poisons, wherein said member is capable of blocking or interrupting polynucleotide synthesis or amplification so as to provide a plurality of [different] single or double-stranded polynucleotides [due to their being in various stages of synthesis or amplification];

(e) denaturing the plurality of [different] single or double stranded polynucleotides to produce a mixture of single-stranded polynucleotides;

- (f) incubating a plurality of said single stranded polynucleotides with a polymerase under conditions which result in annealing of said single-stranded polynucleotides at regions of [identity] homology between the single-stranded polynucleotides and under conditions which promote synthesis of [so as to form] mutagenized [double stranded] polynucleotides, and ;
- (d) expressing at least one [mutant] polypeptide from said mutagenized [double stranded] polynucleotides; wherein the polypeptide possesses a desired characteristic.

3. (Canceled) The method of claim 2, wherein said DNA adduct is a member selected from the group consisting of: UV light; (+)-CC-1065; (+)-CC-1065-(N3-Adenine); a N-acetylated or deacetylated 4'-fluoro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis; trivalent chromium; a trivalent chromium salt; a polycyclic aromatic hydrocarbon ("PAH") DNA adduct capable of inhibiting DNA replication; 7-bromomethyl-benz[ $\alpha$ ]anthracene ("BMA"); tris(2,3-dibromopropyl)phosphate ("Tris-BP"); 1,2-dibromo-3-chloropropane ("DBCP"); 2-bromoacrolein (2BA); benzo[ $\alpha$ ]pyrene-7,8-dihydrodiol-9-10-epoxide ("BPDE"); a platinum(II)halogen salt; N-hydroxy-2-amino-3-methylimidazo(4,5-f)-quinoline; N-hydroxy-2-amino-1-methyl-6-phenylimidazo-pyridine, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerases, chain terminators, and polymerase inhibitors or poisons.



Please add the following new claim:

--11. The method of claim 2, wherein said DNA adduct is a member selected from the group consisting of: UV light; (+)-CC-1065; (+)-CC-1065-(N3-Adenine); a N-acetylated or deacetylated 4'-fluoro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis; trivalent chromium; a trivalent chromium salt; a polycyclic aromatic hydrocarbon ("PAH") DNA adduct capable of inhibiting DNA replication; 7-bromomethyl-benz- $\alpha$ -anthracene ("BMA"); tris(2,3-dibromopropyl)phosphate ("Tris-BP"); 1,2-dibromo-3-chloropropane ("DBCP"); 2-bromoacrolein (2BA); benzo- $\alpha$ -pyrene-7,8-dihydrodiol-9-10-epoxide ("BPDE"); a platinum(II)halogen salt; N-hydroxy-2-amino-3-methylimidazo(4,5-f)-quinoline; N-hydroxy-2-amino-1-methyl-6-phenylimidazo-(4,5-f)-pyridine, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerases, chain terminators, and polymerase inhibitors or poisons.--